

Amendments to the Claims

This listing of claims will replace all prior versions of claims in the application.

1-26 (Cancelled).

27. (Previously Presented) A modified Ca^{2+} -binding polypeptide comprising:

- a) a first chromophore of a donor-acceptor-pair for FRET (Fluorescence Resonance Energy Transfer);
- b) a Ca^{2+} -binding polypeptide with an identity of at least 80% to a 30 amino acid long polypeptide sequence of human troponin C or chicken skeletal muscle troponin C or drosophila troponin C isoform 1; and
- c) a second chromophore of a donor-acceptor-pair for FRET.

28. (Previously Presented) The polypeptide of claim 27, wherein the first chromophore is a fluorescent polypeptide capable of serving as a donor-chromophore in a donor-acceptor-pair for FRET and the second chromophore is a fluorescent polypeptide capable of serving as an acceptor-chromophore in a donor-acceptor-pair for FRET.

29. (Previously Presented) The polypeptide of claim 28, wherein the modified polypeptide is a fusion polypeptide wherein the order of the three linked polypeptides starting from the N-terminus of the fusion polypeptide is a)-b)-c) or c)-b)-a).

30. (Previously Presented) The polypeptide of claim 27, wherein the first chromophore is selected from the group consisting of CFP, EGFP, YFP, DsFP 483, AmCyan, Azami-Green, Cop-Green and As499, particularly wherein the first chromophore is CFP.

31. (Previously Presented) The polypeptide of claim 27, wherein the second chromophore is selected from the group consisting of YFP, DsRed, zFP 538, HcRed, EqFP 611, Phi-Yellow and AsFP 595.

32. (Previously Presented) The polypeptide of claim 31, wherein the second chromophore is YFP.

33. (Previously Presented) The polypeptide of claim 27, wherein the Ca²⁺-binding polypeptide comprises at least one Ca²⁺-binding EF-hand.

34. (Previously Presented) The polypeptide of claim 27, wherein the Ca²⁺-binding polypeptide comprises a polypeptide sequence having at least 60% identity to: (1) amino acids 15 to 163 of chicken skeletal muscle troponin C or (2) amino acids 1 to 161 of human cardiac troponin C or (3) amino acids 5 to 154 of drosophila troponin C isoform 1.

35. (Previously Presented) The polypeptide of claim 27, further comprising glycine-rich linker peptides N-terminal or C-terminal to polypeptide b).

36. (Previously Presented) The polypeptide of claim 27, further comprising a localization signal.

37. (Previously Presented) The polypeptide of claim 36, wherein the localization signal is a nuclear localization sequence, a nuclear export sequence, an endoplasmic reticulum localization sequence, a peroxisome localization sequence, a mitochondrial import sequence, or a mitochondrial localization sequence, a cell membrane targeting sequence.

38. (Previously Presented) The polypeptide of claim 37, wherein the localization signal is a cell membrane targeting sequence mediating localization to pre-or postsynaptic structures.

39. (Currently Amended) The polypeptide of claim 27, which exhibits a ratio change upon Ca²⁺-addition of more than 30%, ~~preferably~~ from 50% to 200%, ~~more preferably~~ from 80% to 180%, ~~and most preferably~~ or from 100% to 150%.

40. (Currently Amended) The polypeptide of claim 27, which has a K_d for Ca^{2+} of from 50 nM to 400 μ M, ~~preferably of from 100 nM to 100 μ M, and most preferably of or from~~ 250 nM to 35 μ M.

41. (Previously Presented) The polypeptide of claim 29, selected from the group consisting of the polypeptides of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 32, 34, and 42, preferably 2, 4, 34, or 42.

42. (Withdrawn) A nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide according to claim 29, preferably a nucleic acid sequence of SEQ ID NO: 1, 3, 33, or 41.

43. (Withdrawn) An expression vector containing the nucleic acid molecule of claim 42, preferably further comprising expression control sequences operatively linked to a nucleic acid encoding a polypeptide, wherein the polypeptide is a modified Ca^{2+} -binding polypeptide comprising:

a) a first chromophore of a donor-acceptor-pair for FRET (Fluorescence Resonance Energy Transfer), wherein the first chromophore is a fluorescent polypeptide capable of serving as a donor-chromophore in a donor-acceptor-pair for FRET;

b) a Ca^{2+} -binding polypeptide with an identity of at least 80% to a 30 amino acid long polypeptide sequence of human troponin C or chicken skeletal muscle troponin C or drosophila troponin C isoform 1;

c) a second chromophore of a donor-acceptor-pair for FRET, wherein the second chromophore is a fluorescent polypeptide capable of serving as an acceptor-chromophore in a donor-acceptor-pair for FRET; and

d) wherein the modified polypeptide is a fusion polypeptide wherein the order of the three linked polypeptides starting from the N-terminus of the fusion polypeptide is a)-b)-c) or c)-b)-a).

44. (Withdrawn) A host cell, particularly a mammalian, non-human cell, inside or outside of the animal body or a human cell outside of the human body, comprising a polypeptide according to claim 29.

45. (Withdrawn) A host cell, particularly a mammalian, non-human cell, inside or outside of the animal body or a human cell outside of the human body, comprising a nucleic acid according to claim 42.

46. (Withdrawn) A host cell, particularly a mammalian, non-human cell, inside or outside of the animal body or a human cell outside of the human body, comprising an expression vector according to claim 43.

47. (Withdrawn) A transgenic animal comprising a polypeptide according to claim 29.

48. (Withdrawn) A transgenic animal comprising a nucleic acid according to claim 42.

49. (Withdrawn) A transgenic animal comprising an expression vector according to claim 43.

50. (Withdrawn) A transgenic animal comprising a host cell according to claim 44.

51. (Withdrawn) A method for the detection of changes in the local Ca^{2+} -concentration comprising the following steps:

- a) providing a cell or a subcellular membraneous fraction of a cell comprising a Ca^{2+} -binding polypeptide according to claim 27;
- b) inducing a change in the local Ca^{2+} -concentration; and

c) measuring FRET between the donor and the acceptor chromophore of the donor-acceptor-pair of said polypeptide according to claim 27, which is indicative of the change in the local Ca^{2+} -concentration.

52. (Withdrawn) The method of claim 51, wherein the cell of step a) is a host cell, particularly a mammalian, non-human cell, inside or outside of the animal body or a human cell outside of the human body, comprising a polypeptide according to claim 29.

53. (Withdrawn) The method of claim 51, wherein the subcellular membraneous fraction is an organelle, in particular a mitochondrion, a peroxisome or a nucleus, or a membrane fraction derived from a membrane-bound organelle, in particular derived from the cell membrane.

54. (Withdrawn) The method of claim 51, wherein the Ca^{2+} -binding polypeptide is targeted to the inner surface of the cell membrane.

55. (Withdrawn) The method of claim 51, wherein step b) is effected by administering an ex- tracellular stimulus, in particular by adding a small chemical compound or a polypeptide to the extracellular side of the host cell.

56. (Withdrawn) A method for the detection of the binding of a small chemical compound or a polypeptide to a Ca^{2+} -binding polypeptide with an identity of at least 80% to a 30 amino acid long polypeptide sequence of human troponin C or chicken skeletal muscle troponin or drosophila troponin C isoform 1, comprising the following steps:

- a) providing a Ca^{2+} -binding polypeptide according to claim 27;
- b) adding a small chemical compound to be tested for binding or a polypeptide to be tested for binding; and
- c) determining the degree of binding by measuring FRET between the donor and the acceptor chromophore of the donor-acceptor-pair of said polypeptide according to claim 27.

57. (Withdrawn) The method of claim 56, wherein the Ca^{2+} -binding polypeptide is derived from human troponin C, and particularly is SEQ ID NO: 4.

58. (Withdrawn) A method of using a polypeptide according to claim 27, comprising the step of detecting changes in the local Ca^{2+} -concentration close to a cellular membrane.

59. (Withdrawn) The method of claim 57, wherein the polypeptide comprises a localization sequence, and particularly comprises a cell membrane targeting sequence, most preferably a cell membrane targeting sequence mediating localization to the cell membrane of pre-or postsynaptic structures.

60. (Withdrawn) A diagnostic composition suitable for the detection of changes in the local Ca^{2+} -concentration close to a cellular membrane, said composition comprising a polypeptide according to claim 27.